

PREVALENCE OF COMMUNITY ACQUIRED METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* NASAL COLONIZATION IN SCHOOL CHILDREN

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ABSTRACT

Methicillin resistant *Staphylococcus aureus* (MRSA) has emerged as a community acquired infection in the past decade. The cause of the increasing incidence of CA-MRSA infection in previously healthy hosts is not completely understood. From October 2012 to November 2012, a total of 388 children ages between 7 years to 12 years, were screened for carriage of MRSA. The overall prevalence of nasal carriage of MRSA and *Staphylococcus aureus* were 4 % and 32 % respectively. About 196 (51%) males and 192 (49 %) females. About 284 (73.0%) were ethnic Bajau, 67(17.0 %) ethnic Malay and remaining other races. MRSA and *S.aureus* colonization was higher in ethnic Bajau 9.0 % and 65.0 % respectively. MRSA colonization was higher in the children ages > 7 to 9 years 7 % but *S.aureus* colonization was higher (50 %) in the children ages > 10 to 12 years. All 146 isolates were susceptible to vancomycin, ciprofloxacin, and gentamicin. MRSA showed moderate resistance to ceftazidime. All isolates showed moderate to high resistance to erythromycin, penicillin and ampicillin respectively.

KEYWORDS: *Staphylococcus aureus*, Colonization, MRSA, CA-MRSA

INTRODUCTION

Staphylococcus aureus is a normal flora of nasal, skin, groin, perineum and axilla. Colonization with *S.aureus* has been identified as an important risk factor for the development of *S.aureus* infections in both community and hospitalized setting [1,2]. The modern era of antimicrobial therapy begin in 1940s with the introduction of penicillin and first beta lactamase such as cephalosporin's and semisynthetic methicillin became available in the late 1950s. First methicillin resistant *Staphylococcus aureus* (MRSA) was described at about same time[3]. The MRSA originally confined to the hospital environment, now MRSA has emerged as a community –acquired infection over the last decade[4-6]. The Community acquired MRSA (CA-MRSA) is different from hospital acquired MRSA from both epidemiological and molecular points of views. Case-definition studies showed that hospital or health-care associated MRSA (HCA-MRSA) and CA-MRSA represented different organisms that produced different clinical syndromes [7,8]. CA-MRSA is an emerging pathogen with an epidemiology and pathogenesis that continue to be defined [9-11]. A recent meta-analysis of studies addressing CA-MRSA colonization in various communities demonstrated a prevalence of 1.3 % [7]. In specific populations, hospital admission within 12 months has been noted to be a risk factor for CA-MRSA carriage; however ,genotypic characteristics of CA-MRSA suggest that most strains have not originated from hospitals [12,13]. The virulence of CA-MRSA may not be limited solely to its resistance to beta lactam antimicrobial drugs. CA-MRSA appears to have a distinct exotoxin genetic armamentarium- in particular Panton-valentine leukocidin (PVL) locus which has been associated with severe infections [14-17].

The aim of this retrospective study was to determine prevalence of CA-MRSA nasal colonization in primary school children.

SUBJECTS AND METHODS

Design

Retrospective study was conducted to determine the CA-MRSA nasal colonization prevalence in primary school children.

Study Area-Setting

The study was conducted in the National Primary school Big Pengalat, Papar District of Sabah, Malaysia. Big Pengalat Primary school with six hundred children

Ethic Clearance

Prior permission from Malaysian Ministry of Education, Director Education, Sabah and ethic clearance was obtained.

Nasal Specimen Collection

Primary school children age 7 to 12 years was involved in the study. Informed consent of parent or guardian was obtained. Subjects on active antibiotic therapy, past two weeks antibiotic therapy or hospitalization were excluded. A demographic detail of subjects was recorded in the study protocol. After all details completion nasal swab were collected with Becton Dickinson culture swabs with Stuart media (BD220093)

Microbiological Identification and Antimicrobial Susceptibility Tests

All specimens were cultured within three hours of collection. Columbia blood agar was used as primary culture, mannitol salt agar(BD), DNase test agar(BD), rapid Latex agglutination test for presumptive coagulase test, Avipath Staph,OD044 kit (Omega Diagnostic,UK) and Gram staining was used for identification. Antibiotic susceptibility test of *Staphylococcus aureus* isolates were tested by Bauer-Kirby disc diffusion techniques (1966) on Muller Hinton agar (BD), according to NCCLS guidelines. Antibiotic disc used in this assay included penicillin(10ug), ampicillin(10ug), erythromycin(10ug), gentamicin(30ug), cefuroxime(30ug) ceftazidime(30ug), rifampin(5ug), ciprofloxacin(5ug), vancomycin(30ug). The antibiotic assay included tests on sensitive control organism with batch of test strain *Staphylococcus aureus* ATCC 25923.. Confirmation of methicillin resistance in *Staphylococcus aureus*-resistance to methicillin was examined by the method described by Barber (1964).

Identification of MRSA was tested by detection of methicillin resistant determinant (*mecA*) product, penicillin binding protein 2 (PBP2) with rapid slide latex agglutination test for identification of MRSA was tested by commercial available test kit (Denka Seiken, Japan)..Molecular typing by pulsed-field gel electrophoreses (PFGE) and Panton-valentine leukocidin (PVL) gene testing were not performed in this study. Data was analyzed by using of Social Sciences Statistical Package (SPSS 16). The difference between values was considered significant when $P < 0.05$ was obtained.

RESULTS

During this study 388 subjects were recruited from Big Pengalat Primary school. The number of subjects in the each age group ranged from 178 >7 to 9 years to 210 for children of ages >10 to 12 years. Of 196 (51%) males and 192 (49%) females. About 284 (73.0%) were ethnic Bajau, 67 (17.0%) ethnic Malay and remaining other racial ethnic groups (table 1). Of total 388 subjects enrolled in the study 146 (37.68 %) were colonized with *Staphylococcus*, Of 146 isolates 125 (32.0%) were methicillin susceptible *Staphylococcus aureus* (MSSA) Of 146 isolates 15 (4.0%) were CA-MRSA and six (1.5%) demonstrated to be methicillin resistant *Staphylococcus* (MRS). The MSSA, CA-MRSA and MRS

nasal colonization in ethnic Bajau was higher (65.0%, 9.0%, and 3.5 % respectively) than in ethnic Malay (MSSA 11.5 %). The MSSA, CA- MRSA and MRS nasal colonization was significantly higher in the children ages >10 to 12 years (50.0%, 3.0 %, and 2.6 % respectively) than in the children children ages > 7 to 9 years (35.5 %, 7.0%, and 1.0 % respectively). (Table 2).

Antimicrobial susceptibility testing of 146 isolates indicated resistance pattern suggestive of CA-MRSA, MRS and MSSA. The detailed susceptibility distribution of various antimicrobials for the isolates is shown in(Table 3). All 146 isolates were susceptible to vancomycin, ciprofloxacin and gentamicin. CA-MRSA showed lower susceptibility to rifampicin and, cefuroxime, but moderate resistance to ceftazidime. All isolates showed moderate to high resistance to ampicillin, penicillin and erythromycin respectively.

Table 1: Demographic of the Study Population: 388 Subjects Analyzed

Variables	Age Group (Years)		Gender		Ethnicity				
	7 to 9	10 to 12	Male	Female	Bajau	Malay	Kadazan	Brunei	Chinese
Number	178	210	196	192	284	67	28	6	3
Percentage %	46	54	51	49	73	17	7	2	1

Table 2: Age, Gender and Ethnicity Distribution of Nasal Carriage of CA- MRSA, MRS and MSSA among School Children n= 146/388 (37.62)

Characteristics	CA-MRSA n=15(4.0)	MRS n=6(1.5)	MSSA n= 125(32.0)
Age Group			
7 to 9	10 (7.0)	2 (1.0)	52 (35.5)
10 to 12	5 (3.5)	4 (3.0)	73 (50.0)
Gender			
Male	5 (3.5)	3 (2.0)	65 (44.5)
Female	10 (7.0)	3 (2.0)	60 (41.0)
Ethnicity			
Bajau	13 (9.0)	5 (3.5)	95 (65.0)
Malay	0		17 (11.5)
Kadazan	2 (1.0)	1 (0.5)	9 (6.0)
Brunei	0		3 (2.0)
Chinese	0	0	1 (0.5)

Figures in Parentheses Indicate Percentage

Table 3: Antimicrobial Susceptibility Pattern of 146 Colonizing CA- MRSA, MRS and MSSA Isolates from School Children

Pathogens	n. Isolates	Antibiotics Resistance %								
		P	AM	E	GN	CXM	CAZ	CIP	RA	VA
CA-MRSA (4%)	15	67	60	74	100	94	60	100	94	100
MRS (1.5%)	6	84	67	84	100	100	34	100	100	100
MSSA (32%)	125	74	75	94	99	100	71	100	100	100

P, penicillin, AM, ampicillin, E, erythromycin, GN, gentamicin, CXM, cefuroxime, CAZ, ceftazidime, CIP, ciprofloxacin, RA, rifampicin, VA, vancomycin

DISCUSSIONS

Our study indicate that the prevalence of nasal MRSA colonization among otherwise healthy children in the Big Pengalat primary school was 4.0 % during the period from October 2012 to November 2012. Compared with a study in Taiwan among children of ages between 2 months and 5 years was 7.3 %.[18]. In the United States, where CA-MRSA is also being increasingly reported the MRSA colonization prevalence for general population appeared to have been relatively low until the year 2002 [19,20]. In a survey involving 9,622 persons conducted between 2001 and 2002, national

S.aureus and MRSA nasal colonization prevalence estimates were 32.4 % and 0.8 %, respectively. For healthy children, the nasal colonization was ranged from 0.22 % to 2.2 % [21,22].

As reported in several pediatric studies; however, and increasing trend in this regard has been noted in certain areas of United States recently [23]. Creech *et al.* reported that nasal MRSA colonization rate among healthy children in Nashville, TN, increased significantly from 0.8 % in 2001 to 9.2 % in 2004. In the United States between 1998 and 2002 researchers reported a MRSA prevalence of 1.3 % [7]. In another study in Taiwan MRSA carriage was 7.8 % among health children from 2005- 2008, with rates ranging from 6.2 % to 9.5 % in different geographic areas [24]. Which was considered to be higher than Taiwanese adult populations surveyed during the same period (3.8 %; $P < 0.0001$) [25]. The CA-MRSA isolates were initially identified in pediatric populations and subsequently reported in adults population [26]. In a prospective observational study in 812 US Army soldiers 3 % were colonized with CA-MRSA and 9 of whom (38 %) developed soft-tissue infections during the study period [27]. Our observations are comparable to other studies.

There are now compelling data suggesting that the pathogenesis of CA-MRSA is unique and distinct form of HA-MRSA. Rather than using the model of HA-MRSA that asserts that there is stepwise progression from MRSA colonization to MRSA infection [28]. Although superficial skin and soft tissue infections remained the most common manifestation of CA-MRSA, severe diseases such as necrotizing pneumonitis, necrotizing fasciitis, osteomyelitis, pyomyositis, septic embolism, and venous thrombosis were not uncommon and previously caused death in healthy children [29,30]. The cause of the increasing incidence of CA-MRSA infection in previously healthy hosts are not completely understood, and the factors influencing CA-MRSA virulence remain an issue of ongoing debate [31-33].

Colonization with *S.aureus* has been identified as an important risk factor for the development of *S.aureus* infections in both community and hospital settings [2,34]. Evidence further suggests that, compared to methicillin-susceptible *S.aureus* (MSSA), colonization with MRSA imposes significantly greater risk for development of subsequent infections [28]. There are several limitations in this study. First, there is no information on the prevalence of CA-MRSA in any past study. Therefore difficult to predict any increase or decrease in prevalence of CA-MRSA colonization in healthy children in this region. Second, our study is retrospective and it lack information if the colonized children developed any superficial or soft tissue infections? Thirdly, molecular typing of CA-MRSA isolates have not been performed and therefore no information if the CA-MRSA isolates belongs to resistant clones of characterized sequence type 59 (ST59) or the clones also possess Panton-Valentine Leukocidin (PVL) genes. Study also lacks information on the family size and information on crowding in the community. Crowding is an independent environmental risk factor that might accelerate CA-MRSA transmission in the community [26]. Further study is needed to know more on CA-MRSA and to address some of the limitations in the study.

CONCLUSIONS

In this study, we observed 4. % healthy school children were colonized by MRSA in the nares from October 2012 to November 2012. Colonization was more common in ethnic Bajau and in children ages >7 to 9 years. Efforts must be made to prevent MRSA spread in the community. Further research is needed to determine the host factors that accelerate spread of MRSA in the community.

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Potential Conflict of Interest

All authors: no conflict.

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